

ULTRASTRUCTURE OF THE HEPATIC SINUSOID OF THE GOAT *CAPRA HIRCUS*

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It is generally agreed that the hepatic sinusoidal lining is discontinuous and that no basement membrane as such exists in the livers of rats (7-9, 14-16, 19, 20, 22), mice (5, 10, 11, 21), dogs (16), rabbits (1, 18), pig embryos (1), frogs (8), developed chick embryos (12), and humans (4, 6, 16). However, several investigators have called attention to patches of "basement membrane-like" material in the space of Disse (5, 10, 18, 20, 21), and actual basement membranes such as are seen around capillaries have been reported in certain pathologic states of the liver (17, 19).

Recently, Wood reported the finding of a complete basement membrane around the sinusoidal blood vessels of calves and contrasted it with the situation in rats, in which no basement membranes were demonstrated when prepared by the same methods in his laboratory (22). Furthermore, he found that the endothelial lining in calf was continuous, though attenuated in many places.

We examined the liver of the goat, which is a ruminant like the calf, and consistently found a definite basement membrane surrounding the sinusoidal blood vessels, and a discontinuous endothelial lining.

METHODS AND MATERIALS

The livers from a total of 6 mature White Angora castrated male goats of healthy appearance were sampled. One animal was sacrificed by electrocution, and specimens were removed within 5 minutes after death. All other specimens were removed from animals under anesthesia (intravenous Nembutal-Veterinary).

In addition, two mature albino rats were sacrificed with ether, and liver samples were taken for light and electron microscopy.

Specimens for electron microscopy were minced fine, fixed in 1 to 2 per cent Veronal-buffered osmium tetroxide at pH 7.4, dehydrated through an alcohol series, and embedded in Maraglas. Ultrathin sections were cut on the LKB ultramicrotome, stained in 3 per cent uranyl acetate or lead citrate, and examined in the RCA EMU-3G microscope.

Specimens for light microscopy were fixed in 10

per cent formalin, processed by usual methods, and stained with hematoxylin and eosin (H and E) and the periodic acid-Schiff reaction (PAS) without diastase.

RESULTS AND DISCUSSION

All specimens embedded in paraffin showed normal-appearing parenchymal cells and uniform thin plates. In addition, thick sections of Maraglas-embedded material stained with toluidine blue or crystal violet did not show any significant pathology. A PAS-positive limiting structure was visible in the sinusoidal areas of all specimens from goats, and the space of Disse was easily seen. These same features were prominent in thick sections of stained Maraglas-embedded material.

The general architecture of the sinusoidal space corresponded very closely to that of the rabbit as diagramed by Steiner (18). The goat had wide perisinusoidal recesses and canals lined by microvilli. Frequently the canals were separated from the bile canaliculus by a solitary desmosome.

The space of Disse varied in width, measuring up to several microns (Fig. 1). Surface microvilli of the hepatic cells were present and ranged from short, blunted processes to long, interlacing ones. At least one type of perisinusoidal cell was seen (Fig. 1). This cell was situated on the sinusoidal basement membrane, separated from it by a low-density space measuring 350 to 600 Å in width, and was frequently associated with bundles of young collagen fibers. The cell itself tended to be long and slender, with smooth borders, or with pinocytotic vesicles along the margins, and had a fibrillar cytoplasm relatively devoid of organelles, although occasionally vesicles were present in abundance. Generally, several of these cells were seen intermittently around a blood vessel, and on two occasions they were noted to contain large lipid droplets. The space of Disse and its contents were all bathed in material indistinguishable from plasma.

There was a continuous basement membrane measuring 350 to 600 Å in width and having the

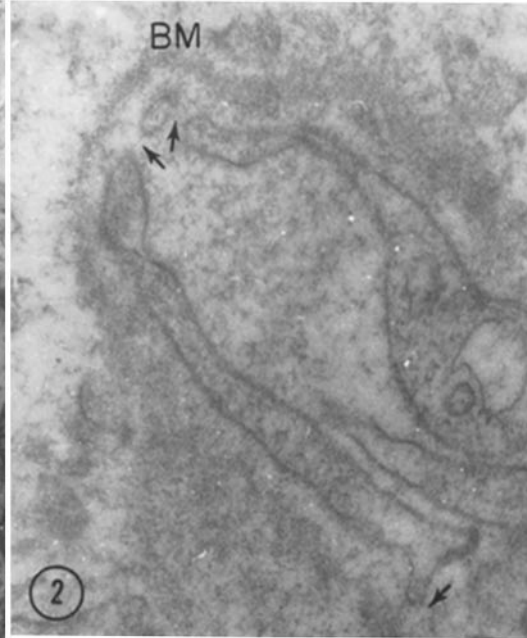


FIGURE 1 Caprine hepatic sinusoid. A blood vessel containing a red blood cell is surrounded by basement membrane (*BM*) and lined by dark endothelial cells (*E*) with cytoplasmic attenuations (arrows). In the narrow space of Disse (*SD*) can be seen hepatic microvilli (*MV*), collagen fibers (*C*), and perisinusoidal cells (*PC*). Lead citrate. $\times 10,000$.

FIGURE 2 High magnification of endothelial gaps (arrows). Note the fibrillar basement membrane (*BM*). Uranyl acetate. $\times 56,000$.

appearance of finely fibrillar feltwork (Figs. 1 and 2). This basement membrane was more or less equidistant between perisinusoidal cell and endothelium, and separated from them on either side by

a low-density space also measuring 350 to 600 Å in width.

The endothelial lining cells contained low-density ground substance, few organelles, and a

variable concentration of vesicles. There were both dark and light cells, the latter being almost completely devoid of organelles, or vesicles, and tending to be situated so as to project into the lumen. However, both forms contained ingested osmiophilic material, and were therefore considered to be granulated and degranulated forms of Kupffer cells. The lining occasionally was made up of two cytoplasmic layers, both confined within the basement membrane. Fenestrations and actual gaps were common (Figs. 1 and 2).

By our methods, the rat hepatic sinusoid was as described by other workers. It had no basement membrane, and endothelial gaps permitted free communication between plasma and liver cells.

The historical background of investigation on the hepatic sinusoids has been well summarized by Aterman (2) and will not be repeated here.

The likelihood that the morphology of sinusoidal

elements is altered by changes in developmental, functional, metabolic, and nutritional states cannot be overemphasized (1, 3, 4, 8, 12, 13, 18, 22). In addition, our results indicate that there may indeed be a species difference in the ultrastructure of hepatic sinusoids as suggested by Wood (22). It remains to be determined whether a basement membrane is a consistent finding in all ruminants.

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In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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